A microscopic view of the cell

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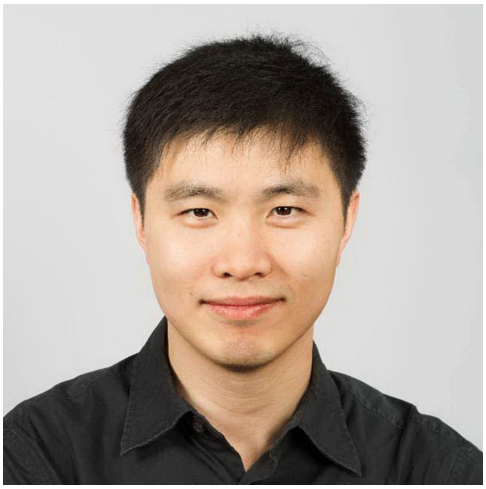
ABSTRACT **Light microscopy has long been an indispensable tool for cell biology research. From biological problems to biological knowledge, there are two more critical links in the light microscopy approach: labeling and quantitative analysis. Therefore, an integrative approach is desirable in order to deal with practical challenges in biological light microscopy.**

In 1665, the word "cell" was first used by Robert Hooke to describe the honeycomb structure of a thin piece of wood cork that he saw under his microscope. Ever since, light microscopy has been an indispensable tool for cell biology research. With its ability to resolve cellular and subcellular structures, analyze their molecular compositions, and follow their dynamics in live specimens, light microscopy has provided us vivid pictures about the inner life of cells. Over the years, the capabilities of light microscopy have continued to expand with many instrumental breakthroughs and innovations. For example, optical sectioning by confocal microscopes provides crisp three-dimensional images. Two-photon fluorescence microscopes allow us to peer deeply into thick tissues. Superresolution microscopes push the spatial resolution from the organelle level to the macromolecule level, turning light microscopy into a new method for architectural analysis of molecular complexes and thus helping to bridge structural biology and cell biology. These technical advancements have greatly expanded the scope of biological problems that can be visualized and investigated using microscopy.



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Microscopy, however, requires more than just the microscope itself. From biological problems to biological knowledge, there are two more critical links: labeling and quantitative analysis (Figure 1). For any structures or activities that do not produce a natural contrast under a microscope, labeling is essential to make them visible. Fluorescent proteins, antibodies, and nucleic acid probes have been widely used to tag specific proteins, DNAs, and RNAs for fluorescence microscopy. Lipids and small-molecule metabolites can be labeled by special chemical bonds or isotopes for Raman detection. Functional probes such as calcium indicators and enzymatic activity reporters shed light on how cells do their everyday work. In all these cases, the information regarding the target is transferred into the positional and optical properties of the labeling reagent, which can be read out by the microscope. To the extreme, in expansion microscopy, the original cellular structure can be entirely removed after the fluorophores are anchored to a polymer matrix.

Although a picture is worth a thousand words, it takes more than just pretty pictures to answer a biological question definitively. Quantitative analysis of microscopic images is crucial in order to objectively extract information such as shape, abundance, colocalization, and movements. Some of these tasks can be accomplished with a few mouse clicks or several lines of scripts in software packages like ImageJ and CellProfiler; in more complicated cases, serious algorithm development and programming may be required. With microscopes becoming more automated and cameras becoming faster, the volume of data has nowadays emerged as yet another challenge as well as an opportunity. Advanced computation such as machine learning has now proven its value in plowing through gigabytes or even terabytes of imaging data.

Because instrumentation, labeling, and analysis are all essential components of microscopy, any challenge in applying microscopy to biological research could be tackled from multiple angles. Let us use a very common issue in fluorescence microscopy as an example.

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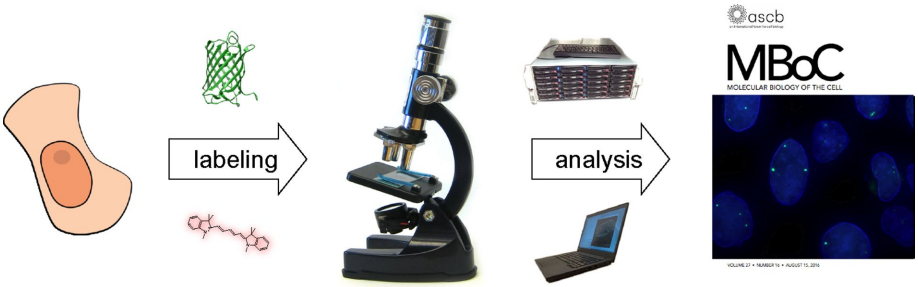

FIGURE 1: A highly simplified scheme for the microscopy approach to cell biology.

When acquiring live confocal stacks of a low-abundance protein, we may find that the signal fades away too quickly because of photobleaching. What can we do then? On one hand, we could move to a light-sheet microscope that prevents out-of-focus regions from light exposure during a three-dimensional scan (Keller and Ahrens, 2015). We could also switch to brighter labels, using the HALO tag (Grimm *et al.*, 2015), SunTag (Tanenbaum *et al.*, 2014), or tandem FP11 tags (Kamiyama *et al.*, 2016), so that the intensity of the excitation light can be lowered without sacrificing the intensity of fluorescence signals. Yet another route is to consider some of the denoising/deconvolution algorithms (Carlton *et al.*, 2010). Using redundant information among camera pixels and across time points and prior knowledge about the structure of interest can dramatically reduce the signal level required for robust statistical interpretation of images. In practice, any one of these approaches could become the optimal choice, depending on the specific biological system studied.

Even more powerful is when the three approaches are integrated. A perfect example is single-molecule switching-based superresolution microscopy, more commonly known by acronyms such as STORM and PALM (Huang *et al.*, 2010). While the challenge is a physical one, that is, the diffraction of a light wave fundamentally limits the spatial resolution of light microscopes, the solution is not just to be found in the optical arena. In addition to a microscope that is sensitive enough to capture snapshots from a single fluorescent molecule, there are two more critical components. First, using fluorophores that can switch from a nonfluorescent to a fluorescent state, we can be achieve a low density of active fluorophores in a densely labeled sample, thus making single-molecule recording possible. Second, by computationally determining fluorophore coordinates from their diffraction-limited snapshots, we can reconstruct a final image with much higher spatial resolution. In this way, the physical diffraction barrier is circumvented without violating the physical principles.

Seamless integration of these three approaches is not easy in practice, though. It is uncommon for a single lab to possess expertise in areas ranging from biology to biochemical labeling to optical instrumentation to computational analysis. Collaboration is extremely helpful here but can sometimes be difficult. People in different fields speak different languages and have different priorities. It can be frustrating when a biologist comes to a microscopy core facility, only to realize that he or she has chosen the "wrong" labeling, which will not match the instrument. It is also a disappointment if a huge pile of images sleeps in the hard drives, simply because the friend in the statistics department cannot be motivated without seeing "new" algorithms coming out of his/her effort. Solving these problems calls for people who have basic understanding of all three fields and can take the initiative to facilitate communication among scientists with diverse expertise. Our systems of funding and evaluation also need to better support and credit people who play "technical service" roles that are actually critical for biological discoveries. In the end, just as microscopy is not just about the microscope, the advancement of microscopy in biology is not only in the hands of microscopists but also in the hands of everyone involved.

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